

**INDUCING DOUBLE OVULATIONS IN BEEF CATTLE VIA SIMULTANEOUS LUTEAL
REGRESSION AND FOLLICLE WAVE EMERGENCE IN A LOW PROGESTERONE
ENVIRONMENT**

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INTRODUCTION

Since cattle are primarily monovular and produce only one calf per year, the cost of production lies mostly in maintaining breeding stock. With proper planning and handling of twin pregnancies, it is estimated that costs per unit of output can be reduced by 24% when beef cows give birth to twins (Guerra-Martinez et al., 1990). However, double ovulations and subsequent twin births in cattle historically have been categorized as an unfortunate event. People with experience in beef and dairy programs know that difficulties can arise with twin births if not managed properly. Being unprepared for a twin birth in beef cattle can lead to many problems including increased calf mortality, malpresentation, increased incidence of retained placentas, and lengthened rebreeding intervals. Dairy cows often experience the same difficulties with twin births; however, the main disadvantage even with a successful twin birth is the decrease in milk production that follows. Producers try to avoid factors that suppress milk production thus twinning is discouraged. Unlike the dairy industry, with beef the main focus is the production of quality offspring.

The negative implications associated with twinning in beef cattle can be reduced or prevented when managed intensively; therefore, the development of a viable system that increases the rate of double ovulations could allow small cow-calf producers to maintain profitability and compete with larger cow-calf operations. Determining the mechanisms which lead to the increasing incidence of twin births in dairy cows would provide an economic advantage considering that each twin

birth costs the dairy industry between \$100 and \$250 (Beereport et al., 1992).

The ability to promote double ovulations in beef cattle yet prevent them in dairy cows would be advantageous.

Earlier studies conducted at The Ohio State University have discovered important pieces to the mechanisms that cause double ovulations in beef cattle and ongoing research with dairy cattle provides useful information on some of the contributing factors that cause double ovulations. Approaches taken in recent studies have successfully caused double ovulations but a practical method that can be implemented in the field has yet to be established. Attempts at using more applicable methods have resulted in multiple ovulations however the capacity to induce the ovulation of just two follicles is limited.

It was found in a serendipitous discovery that when a dominant follicle was aspirated and luteal regression coincided with emergence of the next follicle wave, double ovulations occurred in 47% of the cows (Mussard et al., 2007). Since follicle aspiration is too technical and not cost effective for producers to implement in their own herd, attempts have been made to recreate this environment using a pharmacological approach. Previous studies have used estradiol benzoate (EB) to reset follicular development, which is a more economically viable approach than using the aspiration technique used by Mussard et al. (2007). This approach was successful in inducing follicle wave turnover, and luteal regression was induced coincident with the next follicle wave;

however, double ovulations occurred in <15% of the cows (K. Colliflower, unpublished). It was then hypothesized that the EB treatment used to cause follicle wave turnover attenuated the subsequent follicle stimulating hormone (FSH) increase, resulting in fewer double ovulations. Attempts at supplementing FSH, to circumvent the negative influence of EB, produced variable responses and in some cases a high rate of multiple ovulations (Helsers, 2007). Collectively, the outcome of a series of experiments to test the efficacy of FSH supplementation resulted in the hypothesis that reducing the progesterone concentration prior to the emergence of a new wave of follicular development may be the key to obtaining an increased rate of double ovulations.

In support of the hypothesis that low progesterone concentrations prior to and during emergence of a new cohort of follicles lead to an increased incidence of double ovulations, it has been demonstrated that co-dominance is detected in 35% of lactating dairy cows during the first follicular wave of the estrous cycle, but only 4% of cows in the second wave (Kulick et al., 2001). Concentrations of progesterone are low preceding and during emergence of the first wave, but elevated during emergence of the second wave. Furthermore, in lactating dairy cows that have three follicular waves during their estrous cycle, the ovulatory wave largely develops when progesterone is low. A greater percentage of these animals ovulate two follicles when compared to animals with only two waves of follicular development during the estrous cycle (Bleach et al., 1998). Elevated progesterone concentrations suppressed the ability of these cows to express

their potential to ovulate more than one follicle through suppression of luteinizing hormone concentrations. Therefore, our objective was to study the effect of high and low progesterone concentrations prior to emergence of a new follicular wave on the incidence of double ovulations.

MATERIALS AND METHODS

Animals and Treatments

Estrus was presynchronized in 19 non-lactating Angus and Angus x Simmental beef cows using a seven day synchronization program. All cows were administered 100 µg of GnRH (OvaCyst[®], IVX Animal Health Inc., St. Joseph, MO, USA; im) and an intravaginal progesterone releasing device (CIDR[®], Pfizer Animal Health, New York, NY, USA) was inserted on d -22.5 of the experiment. A single injection of 25 mg of dinoprost tromethamine (PGF; Lutalyse[®], Pfizer Animal Health, New York, NY, USA; im) was given seven days later and CIDR were removed. Animals were visually inspected for signs of behavioral estrus and alternately assigned into two treatments based on time of observed estrus.

On approximately d 13 of the estrous cycle (d 0 of experiment), all cows received a once-used CIDR along with the administration of a single weight adjusted dose of 1 mg/500 kg BW of EB (β-estradiol; Sigma Aldrich Co. St. Louis, MO; 1 mg/mL in benzyl alcohol and arachis oil) to initiate a new follicle wave approximately 3.5 d later. PGF was administered on both d 0 and d 3.5 to establish a low progesterone treatment (LP4, n = 12) or only on d 3.5 to achieve a high

progesterone treatment (HP4, n = 7); from d 0 to 3.5. The CIDR were removed on d 3.5.

Ultrasonography and Estrous Detection

Ovarian structures were monitored and recorded via transrectal ultrasonography using a 7.5 MHz. linear array transducer (Aloka 500V, Corometrics Inc., Wallingford, CT) on d 0 and daily from d 2.5 until ovulation. The location and size of follicles ≥ 5 mm in diameter were recorded at each observation by manual sketches of each ovary. The follicle of greatest diameter, from the new wave of follicles that emerged after EB, is referred to as the dominant follicle (DF). The next largest follicle is referred to as the subordinate follicle (SF). When the DF measured ≥ 12 mm in diameter, a 100 μ g GnRH injection was administered to initiate ovulation. Females were visually inspected for behavioral estrus at 12 hour intervals up until the time of ovulation. All animals detected in estrus before the DF reached 12 mm in diameter did not receive GnRH and were allowed to spontaneously ovulate. Ultrasonography was performed on d 19.5 to determine CL formation.

Determination of Progesterone Concentrations

Blood samples were collected from all females on d 0, 1, 2.5, 3.5, 4.5 and 19.5 to determine blood plasma concentration of progesterone. Blood samples were collected from the jugular vein into 10 mL Vacutainer™ tubes containing an anticoagulant (EDTA). Samples were immediately placed on ice after collection and centrifuged within 1 hour. All samples were centrifuged at 1,500 x g for 20

minutes and plasma transferred to separate 5 mL polyethylene tubes. Samples were stored at -20°C until concentrations of progesterone were determined using a RIA kit (Coat-a-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA), as described previously for our laboratory (Burke et al., 2003).

Statistical Analysis

Two animals from the LP4 treatment did not undergo follicle wave turnover and were not included in statistical analysis. Emergence of the new follicular wave was determined by the appearance of a new follicle measuring 5 mm in diameter. Ovulatory follicle size was defined as the diameter of the follicle the day before its disappearance from the ovary and day of ovulation was determined as the day the follicle disappeared from the ovary. Discrete hormonal and follicular characteristics were analyzed by ANOVA using the MIXED procedures of SAS (Version 9.1, SAS Institute Inc., Cary, NC). Progesterone and follicular changes over time were compared by ANOVA using the MIXED procedures of SAS with repeated measures to test effects of treatment, time, and the treatment by time interaction. Specific mean comparisons were made using the SLICE option of SAS following detection of a significant effect in the main model. All data are expressed as the actual mean \pm SEM. Statistical significance was considered to be $P < 0.05$.

RESULTS

A new wave of follicles was observed in 17 of 19 cows (HP4, 7/7; LP4, 10/12) and the mean day of follicle wave emergence did not differ between the HP4 and

LP4 treatments ($d\ 3.6 \pm 0.3$ vs. $d\ 3.7 \pm 0.2$). Concentrations of progesterone in the HP4 treatment were significantly greater from d 1 through d 4.5 when compared to the LP4 treatment ($P < 0.01$, Figure 1).

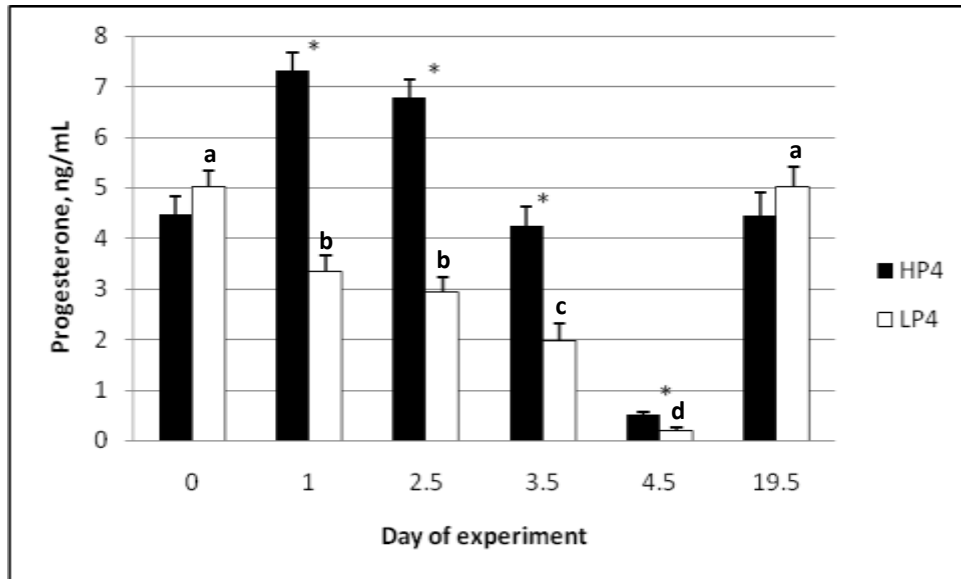


Figure 1. Circulating concentrations of progesterone (means \pm SEM) in cows not treated with PGF d 0 of experiment (HP4) or in cows treated with PGF d 0 of experiment (LP4). D 0 represents the day of EB injection, insertion of used CIDR, and +/- PGF. A treatment x day interaction was detected between the HP4 and LP4 treatments [an asterisk (*) indicates that means within day differ, $P < 0.01$]. Different letters indicate that means within the LP4 treatment differ among days, $P < 0.01$.

Ultrasonography showed a decrease in CL diameter from d 0 to d 2.5 in all cows within the LP4 treatment, indicative of PGF-induced luteolysis. Progesterone analyses confirmed luteolysis showing a significant decrease in mean progesterone concentrations, from 5.0 ± 0.3 ng/mL on d 0 to 3.3 ± 0.3 ng/mL 24 hours later ($P < 0.01$, Figure 1). No differences were detected between treatments in DF diameter over the 5 days following emergence ($P = 0.65$, Figure 2).

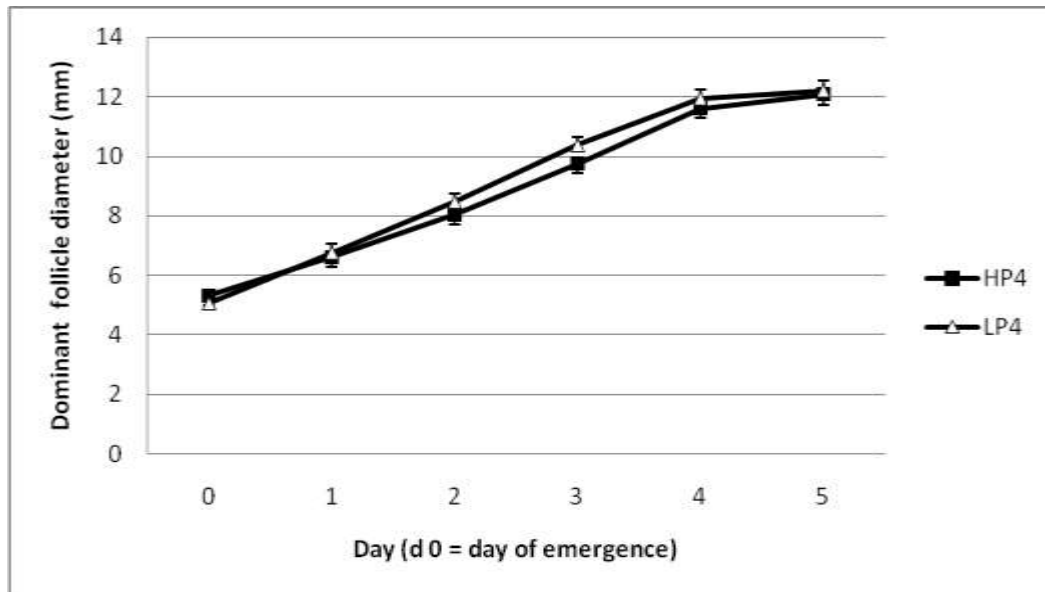


Figure 2. Mean follicular diameter for the HP4 and LP4 groups from d 0 to d 5 after emergence.

The proportion of animals showing estrus in the HP4 treatment and LP4 treatment was 3/7 and 5/10, respectively. All animals ovulated a single follicle with the exception of one animal in each treatment that ovulated two follicles. The mean follicle diameter at ovulation (12.0 ± 0.2 mm vs. 11.8 ± 0.4 mm, $P=0.71$) and mean interval to ovulation (d 6.0 ± 0.0 d vs. d 5.5 ± 0.2) did not differ between the HP4 and LP4 treatments, respectively.

DISCUSSION

The aim of this study was to determine if decreased concentrations of progesterone during the period of time in which a new cohort of follicles was emerging would increase the incidence of double ovulations. The design of the current approach was based on previous work done in our lab to find a practical method to induce double ovulations. Previous work utilized the “day 13 follicle

wave turnover” approach to resetting follicular growth using estradiol benzoate as described by Burke et al. (2002). This method was chosen because the time of follicle wave emergence can be estimated with great precision and can be expected to occur approximately 3.5 d after estradiol benzoate is administered. Work by Helser et al. (2007) was focused on causing a new wave of follicular development concurrently with luteal regression. In these studies, it was found that emergence was highly synchronous between most animals, thus, we implemented the same day 13 follicle turnover strategy in the current study.

In order to achieve a low progesterone treatment, it was necessary to induce luteal regression 3.5 d prior to expected emergence. A subsequent decrease in circulating progesterone following administration of PGF was our goal; however progesterone is a requirement for estradiol benzoate to be effective. It has been shown that the effectiveness of estradiol benzoate to induce follicle wave turnover is greatly reduced when luteolysis occurs in less than 48 hours after the administration of estradiol benzoate (Burke et al., 1997). Since luteolysis was induced with PGF to form a low progesterone treatment, yet the presence of progesterone is required for the use of estradiol benzoate, a once-used CIDR was inserted to supply cows with a low level of progesterone. In the present study, follicle wave emergence occurred in 17 of 19 cows and the time of emergence was consistent between both treatments. This success can be attributed to providing an adequate progesterone environment for the efficacy of

estradiol benzoate and also to the utilization of the highly predictable day 13 follicle wave turnover model.

Although our methods were successful at precisely inducing follicle wave emergence and altering progesterone concentrations up to the time of emergence, this approach did not result in the selection and ovulation of more than one ovarian follicles. Our hypothesis was that animals with reduced progesterone concentrations during the time preceding emergence would exhibit co-dominance and hence, double ovulation. In our model, concentrations of progesterone were greater in the HP4 than LP4 treatment prior to emergence as a result of an active CL in the HP4 treatment. The only source of progesterone in the LP4 treatment was from the CIDR because of PGF-induced luteolysis of the CL on d 0. The concentration of progesterone on the day of follicular emergence was similar between the aspiration method (Burke et al. 2003) and the present study, 2.7 ± 0.2 vs. 2.0 ± 0.3 ng/mL, respectively. However, with the aspiration approach that previously resulted in 47% of animals with double ovulations, progesterone concentrations increased from relatively low levels over the 2 to 4 days before emergence, whereas in the present study, progesterone decreased from high, mid-luteal concentrations present on d 0 over this time period. We speculate that the pattern of progesterone preceding follicular emergence might influence the incidence of double ovulations. Therefore, alternative approaches to regulate progesterone during the pre-emergent period are needed for the development of a practical method to consistently induce double ovulations.

Perhaps this would be most easily achieved by manipulation of the first wave of follicles, when endogenous concentrations of progesterone are inherently low, rather than controlling follicular development during the middle of the estrous cycle, when progesterone concentrations are maximal.

Estradiol benzoate was used in the present and previous double ovulation studies as a means to avoid using follicle aspiration, which is an expensive and impractical method for field use. The use of estradiol benzoate is a highly predictable method of causing follicle wave turnover and a new wave to emerge; however, it does delay the release of FSH (Burke et al., 2003). There are many possible approaches that might be used to circumvent this problem and some have already been tested. The use of supplemental FSH to overcome this inhibition by estradiol benzoate was described previously in the introduction, but the variable responses limit the usefulness of this approach to induce double ovulations (Helser, 2007). Different dosages of estradiol benzoate have not been tested with the current experimental design. Bogacz et al. (1999) identified the optimal dose as 1 mg/500 kg BW to induce a new follicular wave in mature beef cows on d 13 of the estrous cycle, which is the dose used in the present experiment. Perhaps an alternative dose would be more effective in the programs designed to induce double ovulation. Further research would be necessary to test this idea. Finally, an alternative method to synchronize follicle emergence (e.g. GnRH) might be beneficial.

CONCLUSIONS

Reduction of progesterone concentrations that preceded emergence did not increase double ovulation rate in cattle and we therefore reject our hypothesis for the present experiment. It is possible that the extent of reduction in progesterone, or the temporal pattern of its secretion over the days preceding emergence may have inhibited expression of co-dominance. Further research that results in more drastic reductions in progesterone concentrations is necessary to determine the role, if any, of progesterone concentrations in this process.

IMPLICATIONS

The economic crisis that beef and dairy producers are experiencing with the high price of inputs and the low value of meat and dairy products is forcing these industries to look even more closely at alternative ways to reduce cost and increase their bottom line. This economic situation has the potential to increase the interest in the mechanisms associated with double ovulations and twinning. As this interest from producers brings together both dedicated scientists and financial support, the opportunity to produce or prevent twins will prove to be beneficial for beef and dairy producers, as well as, the scientific community.

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